

Protein Biomarkers Can Distinguish Symptomatic from Asymptomatic Degenerative Lumbar Intervertebral Discs

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Introduction: The prevalence of chronic low back pain (LBP) can exceed 30% in some populations. While pathomechanisms for the development of LBP may vary between individuals, intervertebral disc degeneration (IVDD) is consistently noted. However, while IVDD is commonly observed in patients with chronic LBP, many patients with significant IVDD are not symptomatic for LBP. It is unclear as to why some patients develop symptoms, while other individuals with similar degrees of disc degeneration remain asymptomatic. It is suspected that the development of symptomatic IVDD is a multifactorial process that is affected by patient, tissue, cellular, and molecular factors that contribute to pathomechanisms that result in pain. This study was designed to determine if differences in the *ex vivo* release of inflammation-, degradation-, and growth factor-related proteins by IVD tissues recovered from symptomatic (SYM) clinical patients and asymptomatic (ASYM) tissue donors could be identified for a given IVDD severity grade. It was hypothesized that SYM IVD tissues would release significantly higher levels of pro-inflammatory and pro-degradative proteins compared to ASYM IVD tissues when matched for IVDD severity grade.

Methods: Donor and Surgical Patient IVD Specimen Collection and Processing: With IRB approval (IRB#2010692) and informed patient consent, IVD tissues were recovered from SYM patients undergoing surgery for IVDD (n=184 patients, mean age 55.8y, 105F). With consent recorded in a legal permit under the Uniform Anatomical Gift Act, IVD tissues were recovered from qualified ASYM tissue donors (n=20 patients, mean age 51.8y, 10F) without a reported history of back pain. IVDD was quantified using the Thompson grade gross assessment (ASYM) or Pfirrmann MRI grading system (SYM) using pre-surgical images (T/P-grade). Tissue explants for each SYM patient, and annulus fibrosus (AF) and nucleus pulposus (NP) tissue explants from ASYM donors were created and cultured for 3 days. On day 3 the media were collected for biomarker analysis, tissues were weighed, and half the SYM tissue explant was processed for histological assessment. **Histological Assessment of SYM IVD Tissue Type:** Each SYM IVD specimen was formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and stained using H&E, Toluidine blue, and Picrosirius red. Histological classification of tissue type was performed (AF or NP). S-IVD specimens that were a mixture of tissue types (AF, NP, cartilage endplate, and/or bone) were excluded from analysis. **Media Analysis:** Media samples were tested for IL-6, IL-8, Gro- α , MCP-1, MCP-3, MIP-1 α , MIP-1 β , RANTES, TNF- α , PDGF-AA, VEGF, MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13 using Luminex assays. **Statistical analysis:** Media biomarker concentrations were standardized to the wet weight of each explant and natural log transformed. Multivariable linear models (LMs) were created for each biomarker, and SYM AF or NP tissues were compared to ASYM AF or NP tissues with adjustment for patient age, sex, obesity status, and their interactions. Two-sided significance was set at $p \leq 0.05$. Interaction plots of predicted biomarker values were used to interpret LM results.

Results: Inflammatory Cytokines/Chemokines: SYM AF and NP tissues released significantly ($p \leq 0.037$) higher levels of GRO- α (T/P-grade 5), IL-1Ra (T/P-grades 2/3), MCP-1 (T/P-grade 5) and RANTES (All T/P-grades) compared to ASYM AF and NP tissues, while accounting for sex, age, and obesity status (Figures 1A and 1B). Additionally, SYM NP tissues released significantly ($p \leq 0.039$) higher levels of IL-1Ra (T/P-grade 5), IL-6, IL-8, MIP-1 β (T/P-grades 2/3 & 5), and MIP-1 α (All T/P-grades), compared to ASYM NP tissues. **Matrix Metalloproteinases:** SYM AF and NP tissues released significantly ($p \leq 0.019$) higher levels of MMP-2 and MMP-13 (T/P-grades 2/3 & 4), and significantly ($p \leq 0.023$) lower levels of MMP-3 (T/P-grade 2/3) and MMP-8 (all T/P-grades), compared to ASYM AF and NP tissues (Figures 2A and 2B). Further, SYM AF tissues released significantly ($p \leq 0.037$) higher levels of MMP-13 (T/P-grade 5), and significantly ($p = 0.010$) lower levels of MMP-3 (T/P-grade 4), compared to ASYM AF tissues. SYM NP tissues released significantly ($p \leq 0.037$) higher levels of MMP-1 (T/P-grade 5) compared to ASYM NP tissues. **Tissue Inhibitors of Metalloproteinases and Growth Factors:** ASYM AF and NP tissues released significantly ($p \leq 0.050$) higher levels of TIMP-2, TIMP-3, and TIMP-4 (all T/P-grades) and TIMP-1 (T/P-grades 2/3) compared to SYM AF and NP tissues (Figures 3A and 3B). SYM NP tissues released significantly ($p \leq 0.04$) higher levels of PDGF-AA and VEGF (T/P-grades 2/3 & 4) compared to ASYM NP tissues. SYM AF tissues released significantly ($p \leq 0.022$) higher levels of PDGF-AA (T/P-grades 2/3) compared to ASYM AF tissues.

Discussion: The data from this study indicate that degenerative disc tissues from symptomatic patients are associated with significantly higher release of inflammatory proteins and specific MMPs, and significantly lower release of TIMPs, compared to degenerative disc tissues from asymptomatic individuals with similar morphologic degeneration. These findings suggest that pro-inflammatory signaling and degradative enzyme activity, especially in the earlier stages of disease, may be important distinguishing factors in the development of symptomatic IVDD in patients. Interestingly, the majority of significant differences in biomarkers noted for SYM versus ASYM IVDs involved the nucleus pulposus when compared to the annulus fibrosis, suggesting that pain-related processes may be driven primarily from the nucleus pulposus rather than the annulus fibrosis. However, further study is required to determine if these changes in IVD protein release profiles are causative or are a consequence of symptomatic IVDD. Ongoing studies in our lab are aimed at further characterization of symptomatic and asymptomatic IVDs to determine direct links to development of symptomatic IVDD that may serve as biomarkers for clinical application.

Significance: The quantification and comparison of tissue protein release profiles indicate significant differences between IVD tissues recovered from symptomatic patients and asymptomatic donors that could be related to the development and/or severity of symptomatic IVDD. Relating these differences to the development and progression of symptomatic IVD degeneration may allow for the development of novel diagnostic, preventative, and treatment methodologies for patients with debilitating lower back pain.